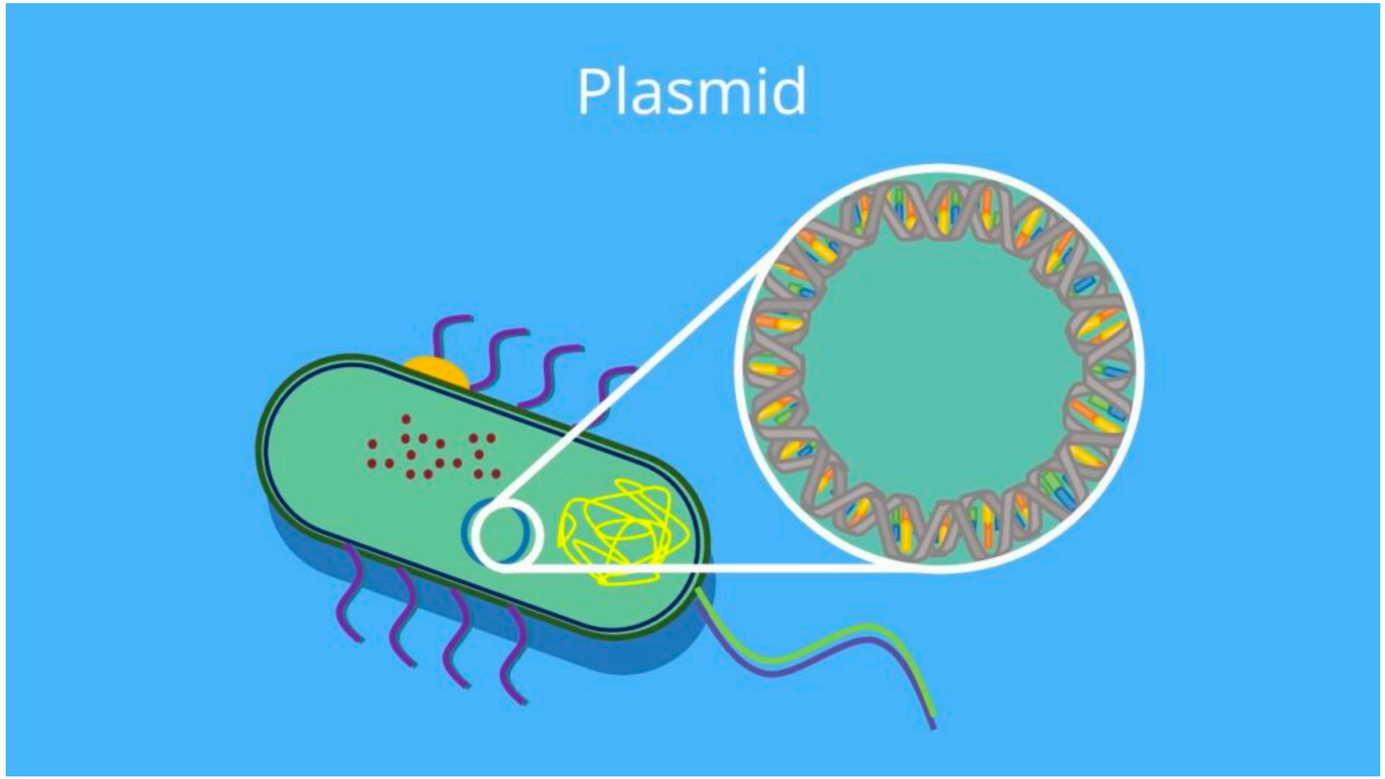
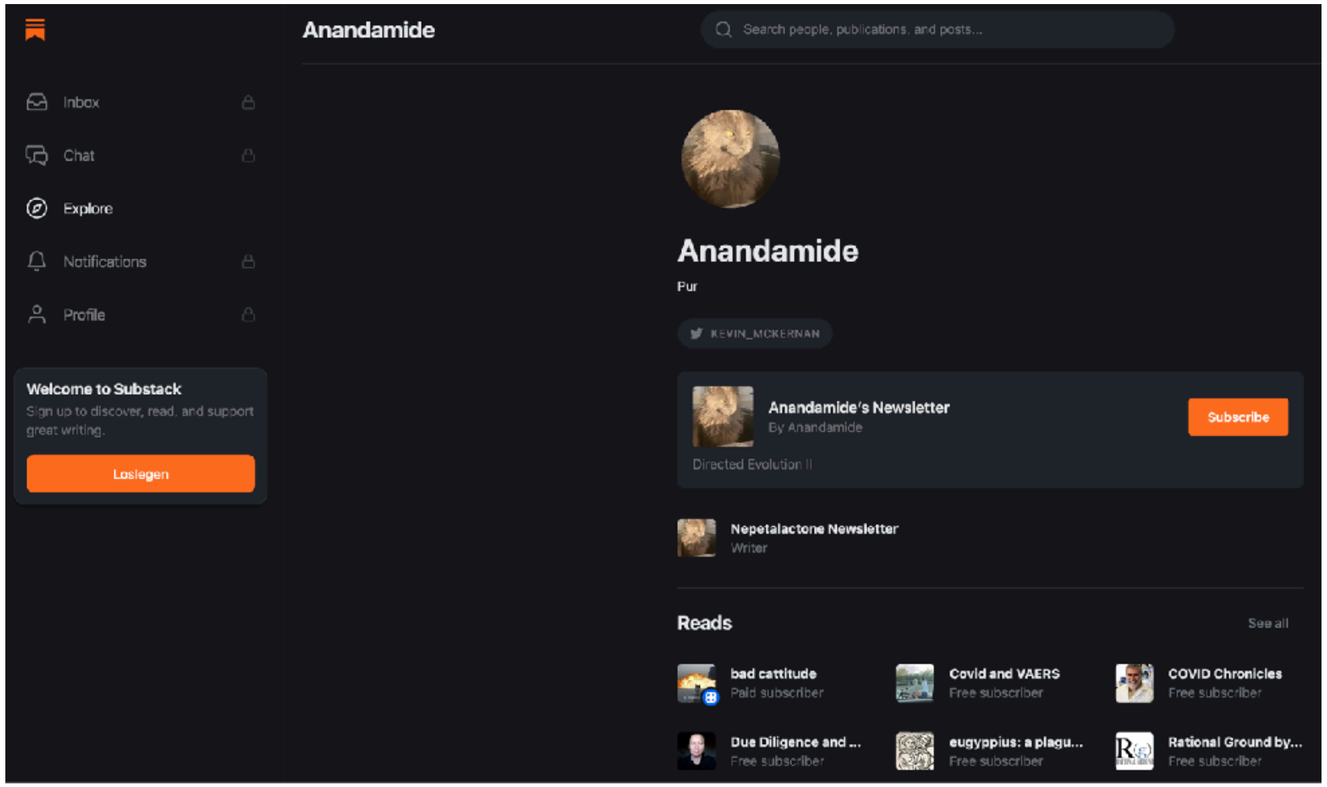
Plasmide in mRNA-Vakzinen



Ausgangslage

- Kontamination der RNA-Vakzine mit bakteriellen Komponenten
- Nachgewiesen: dsDNA (Plasmide)
- Damit möglich: Fortgesetzte Expression (Spike-Bildung) & DNA-Integration
- Damit möglich: Endotoxine (LPS)
- Damit hinfällig: Zulassung

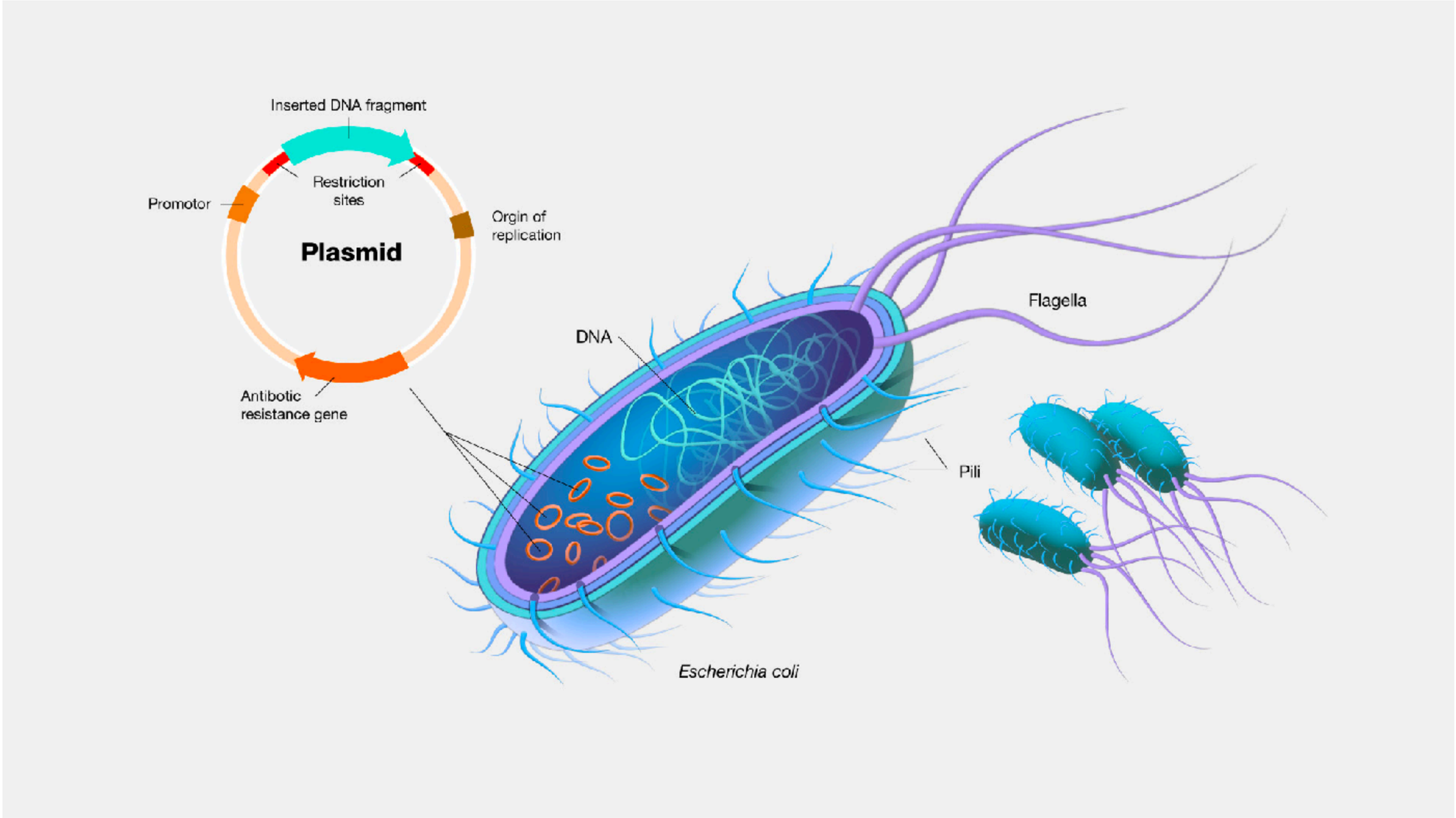
Ausgangslage



https://substack.com/profile/32722070-anandamide

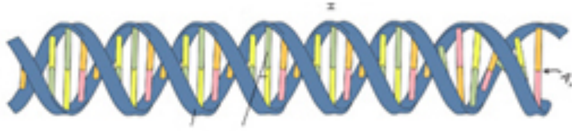
Wer oder was sind Plasmide?

Plasmide

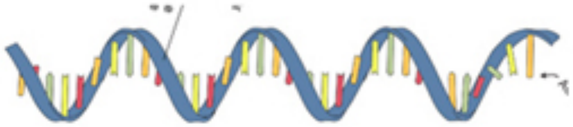

Inserted DNA fragment
Restriction sites
Promotor
Orgin of replication
Plasmid
Antibotic resistance gene
DNA
Flagella
Pili
Escherichia coli

Plasmide

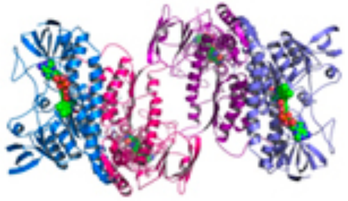
DNA



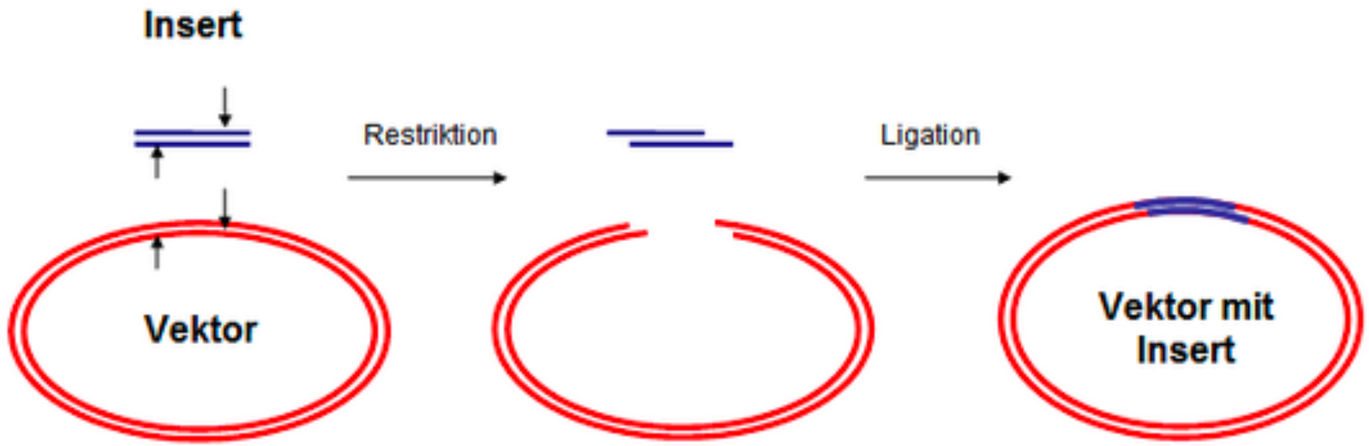
RNA

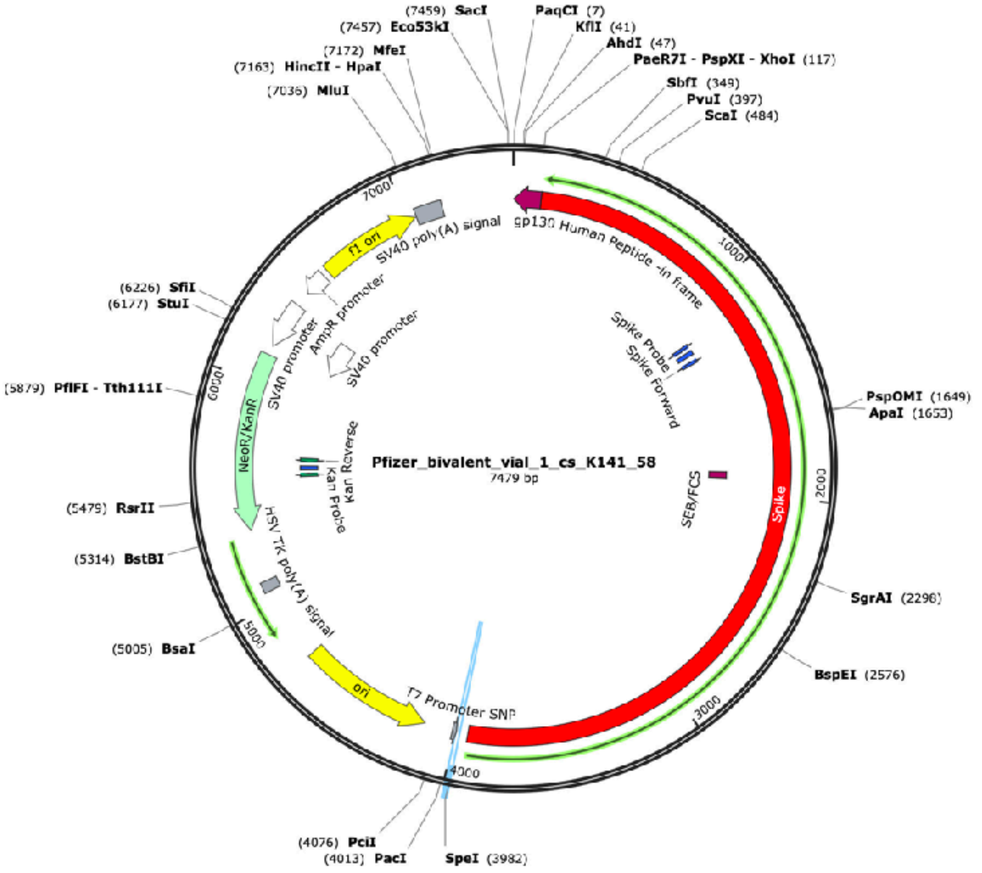


PROTEIN

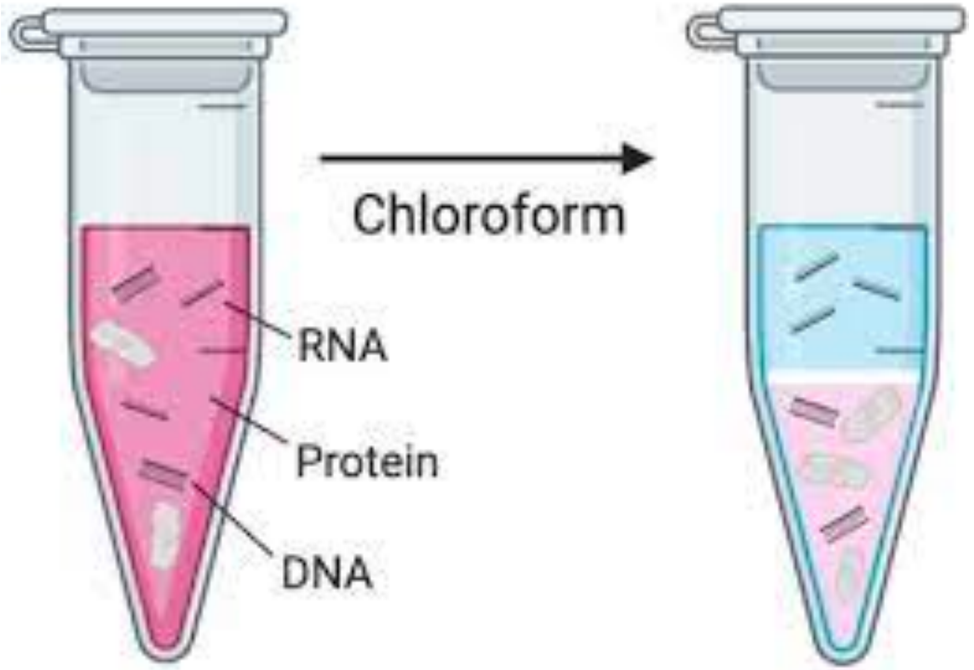


Plasmide


Insert
Restriktion
Ligation
Vektor
Vektor mit
Insert


(7459) SacI
PaqCI (7)
(7457) Eco53kI
KflI (41)
AhdI (47)
(7172) MfeI
PaeR7I - PspXI - XhoI (117)
(7163) HincII - HpaI
SbfI (349)
(7036) MluI
PvuI (397)
ScaI (484)
7000
f1 ori
SV40 poly(A) signal
sp130 Human Peptide -in frame
1000
(6226) SfiI
(6177) StuI
AmpR promoter
SV40 promoter
SV40 promoter
Spike Probe
Spike Forward
(5879) PflFI - Tth111I
6000
NeoR/KanR
PspOMI (1649)
ApaI (1653)
Pfizer_bivalent_vial_1_cs_K141_58
7479 bp
Kan Reverse
Kan Probe
SEB/FCS
(5479) RsrII
Spike
2000
(5314) BstBI
HSV TK poly(A) signal
SgrAI (2296)
5000
(5005) BsaI
ori
T7 Promoter SNP
BspEI (2576)
3000
4000
(4076) PciI
(4013) PacI
SpeI (3982)

Plasmide



Genomintegrität

- Keine Qualitätssicherung durch Sequenzierung der Chargen
- Keine Expressionsanalyse der Chargen
- Pseudouridin als Fehlerquelle (1 von 4000 Nukleotiden, entspricht 5 - 8.5 Mio SNPs/Impfung)

characterisation data on the omicron variant, which is considered mandatory to guarantee safety of the product.

In response, the Applicant has provided characterisation data for the Omicron (BA.1) variant. The package includes confirmation of primary structure, 5'-Cap structure, higher order structure and biological activity. Essentially, the same methods as those used for characterisation of the original variant have been applied. It is noted that primary structure analysis by NGS has been excluded. However, the HPLC-UV and LC-MS/MS studies are found sufficient to confirm the primary structure.

Biological activity is confirmed by western blot analysis and cell-free in vitro translation. This is found acceptable. However, some details for the western blot analysis are lacking and the identity of the observed bands are not clear. It is recommended that the applicant provide this information post-approval.

- The expressed protein size for BNT162b2 Omicron (B.1.1.529) DS is evaluated by western blot. The Applicant claims that the protein size is consistent with the expected size of the translated protein. However, the theoretical protein sizes of the mature protein and variants thereof are not presented in the dossier. This information should be provided, and the bands observed by WB should be assigned. In addition, the antibody used for western blot should be further described, i.e., it should be stated if it targets the S1 or S2 domain of the protein. The

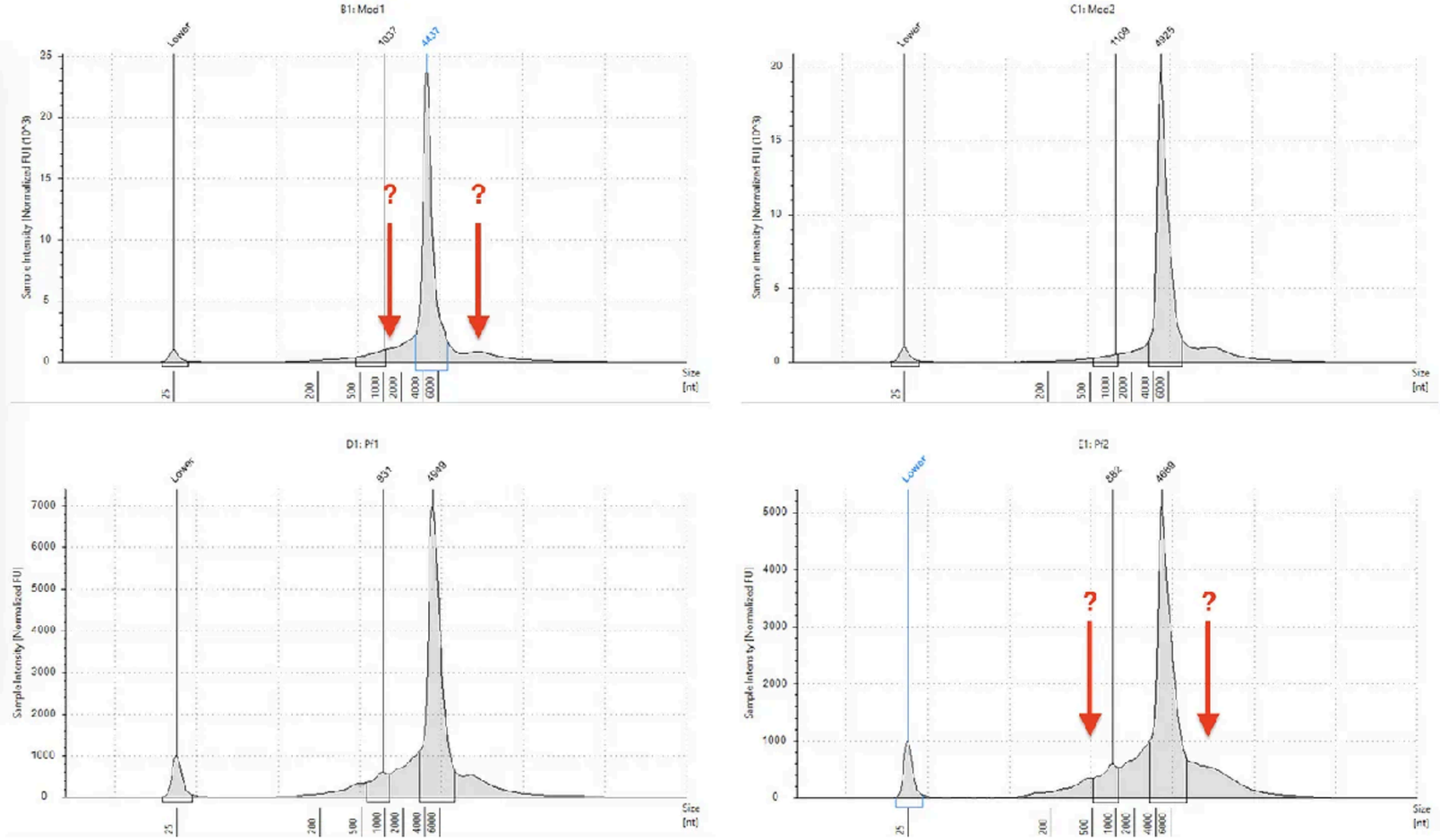


Figure 1. Agilent Tape Station Electrophoresis of the Bivalent vaccines. Moderna mRNA-1273.214 (Top) and the Pfizer bivalent vaccine (Bottom).

Plasmide & Vaccines

https://anandamide.substack.com/p/curious-kittens

- Moderna: 1:3000 Plasmid-DNA/modRNA
- Pfizer: 1:350 Plasmid-DNA/modRNA

Residual DNA Template

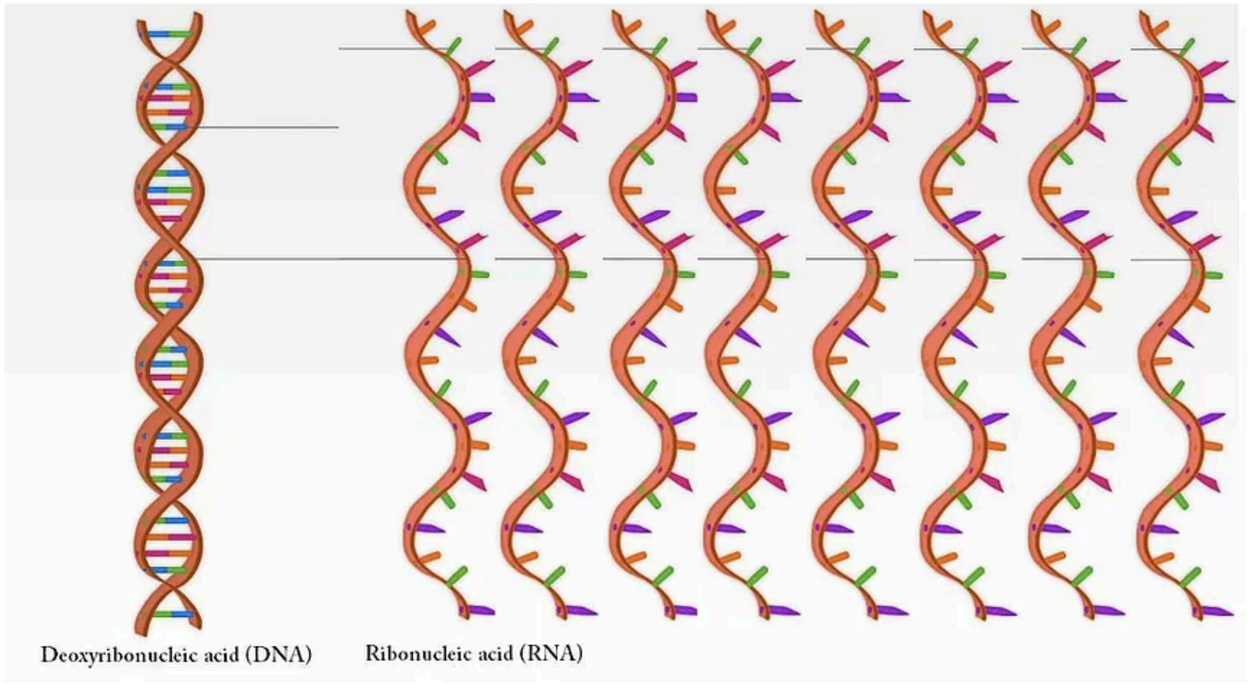
Residual DNA template is a process-related impurity derived from the linearized DNA template added to the in-vitro transcription reaction. Residual DNA template is further controlled through routine testing using the analytical procedure described in 3.2.S.4.2 Quantitative Polymerase Chain Reaction(qPCR) and the BNT162b2 drug substance specification as described in 3.2.S.4.1 Specification. Results are shown in Table S.3.2-1 for process validation batches manufactured to date

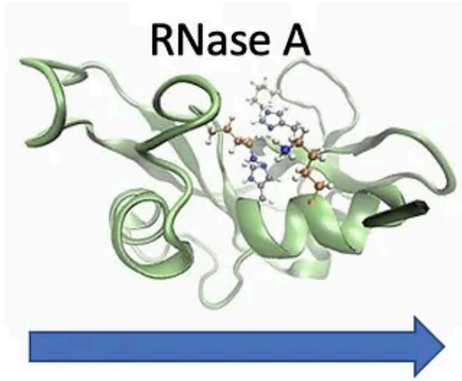
Table S.3.2-1 Residual DNA Template Results for Clinical, Initial Emergency Supply and Process Performance Qualification COVID-19 Vaccine BNT162b2 Drug Substance Batches (Andover)

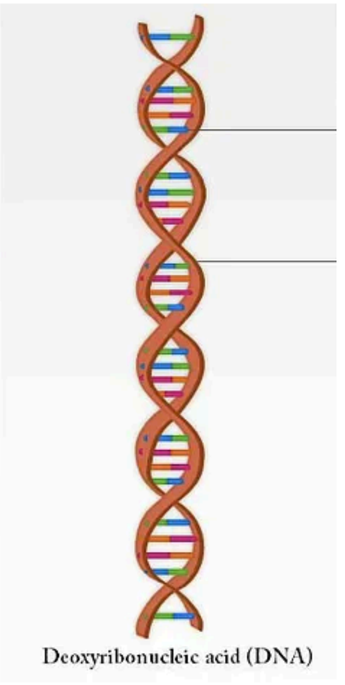
Batch		20Y513C101	20Y513C201	20Y513C301	20Y513C401	20Y513C501
Sample	Acceptance Criteria	Results				
Drug Substance	≤ 330 ng DNA / mg RNA	17	29	10	23	211

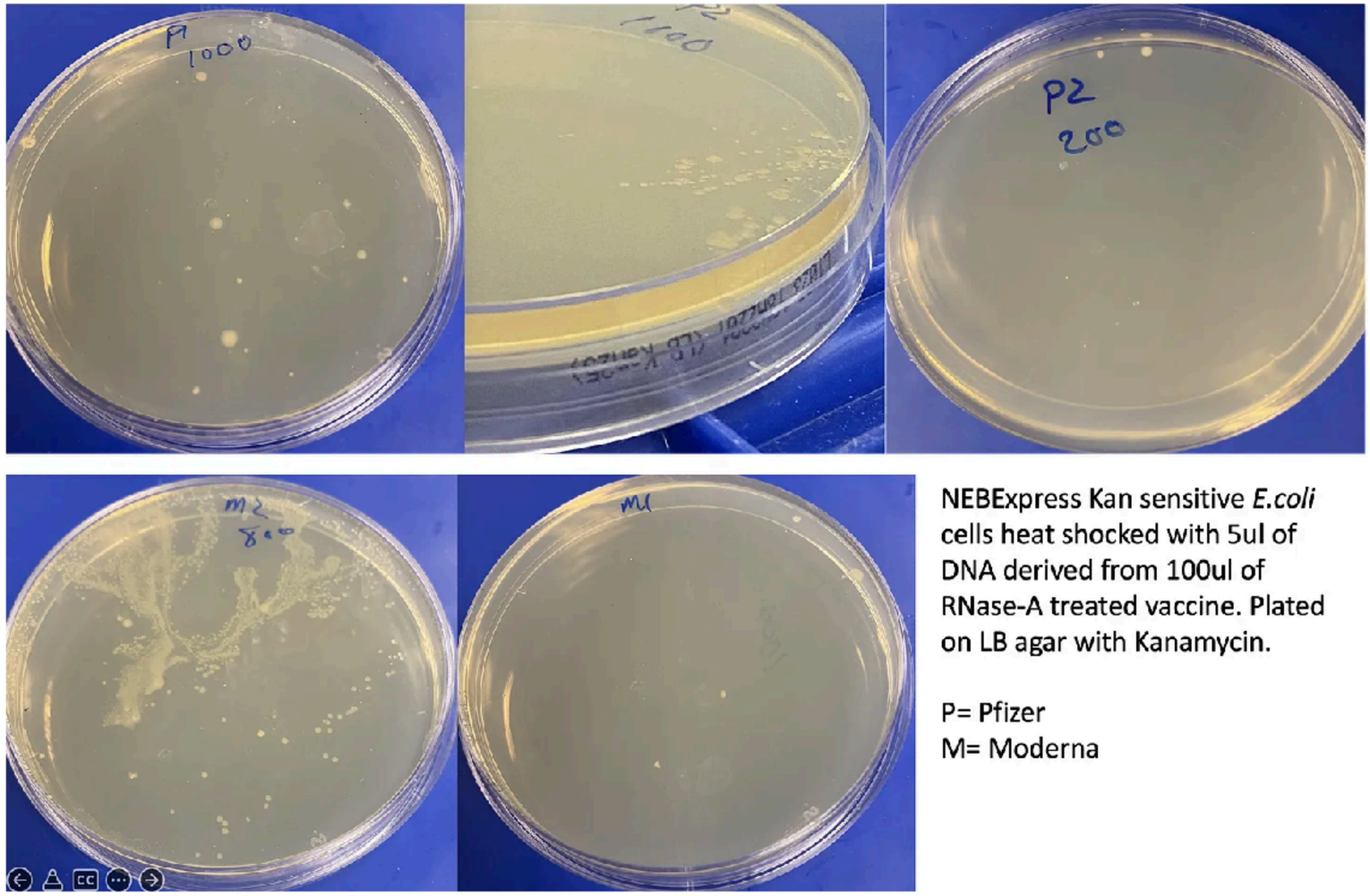
Abbreviations: DNA = deoxyribonucleic acid; RNA = ribonucleic acid

Probe aufs Exempel









NEBExpress Kan sensitive *E.coli* cells heat shocked with 5ul of DNA derived from 100ul of RNase-A treated vaccine. Plated on LB agar with Kanamycin.

P= Pfizer
M= Moderna

Figure 3. Transformation of NEBExpress Kan sensitive E.coli cells. Plates were grown at 37C for 48 hours. 200ul and 800ul of SOC were plated in order to capture the entire transformation on 1 plate.

Conclusions

Previous RNA-Seq based estimates of the double stranded DNA contamination in the vaccines significantly under reported the magnitude of the contamination. Using qPCR and electrophoresis, we demonstrate the dsDNA contamination levels are 100 fold higher and imply trillions of DNA molecules per dose. The DNA contamination ranges from 8.19-11.3 ng/ul with 23-55ng/ul of mRNA. This equates to 20-35% of the nucleic acid in each vaccine being expression vector. This is several orders of magnitude over the the EMAs limit of 330ng/mg.

Residual DNA Template

Residual DNA template is a process-related impurity derived from the linearized DNA template added to the in-vitro transcription reaction. Residual DNA template is further controlled through routine testing using the analytical procedure described in 3.2.S.4.2 Quantitative Polymerase Chain Reaction(qPCR) and the BNT162b2 drug substance specification as described in 3.2.S.4.1 Specification. Results are shown in Table S.3.2-1 for process validation batches manufactured to date

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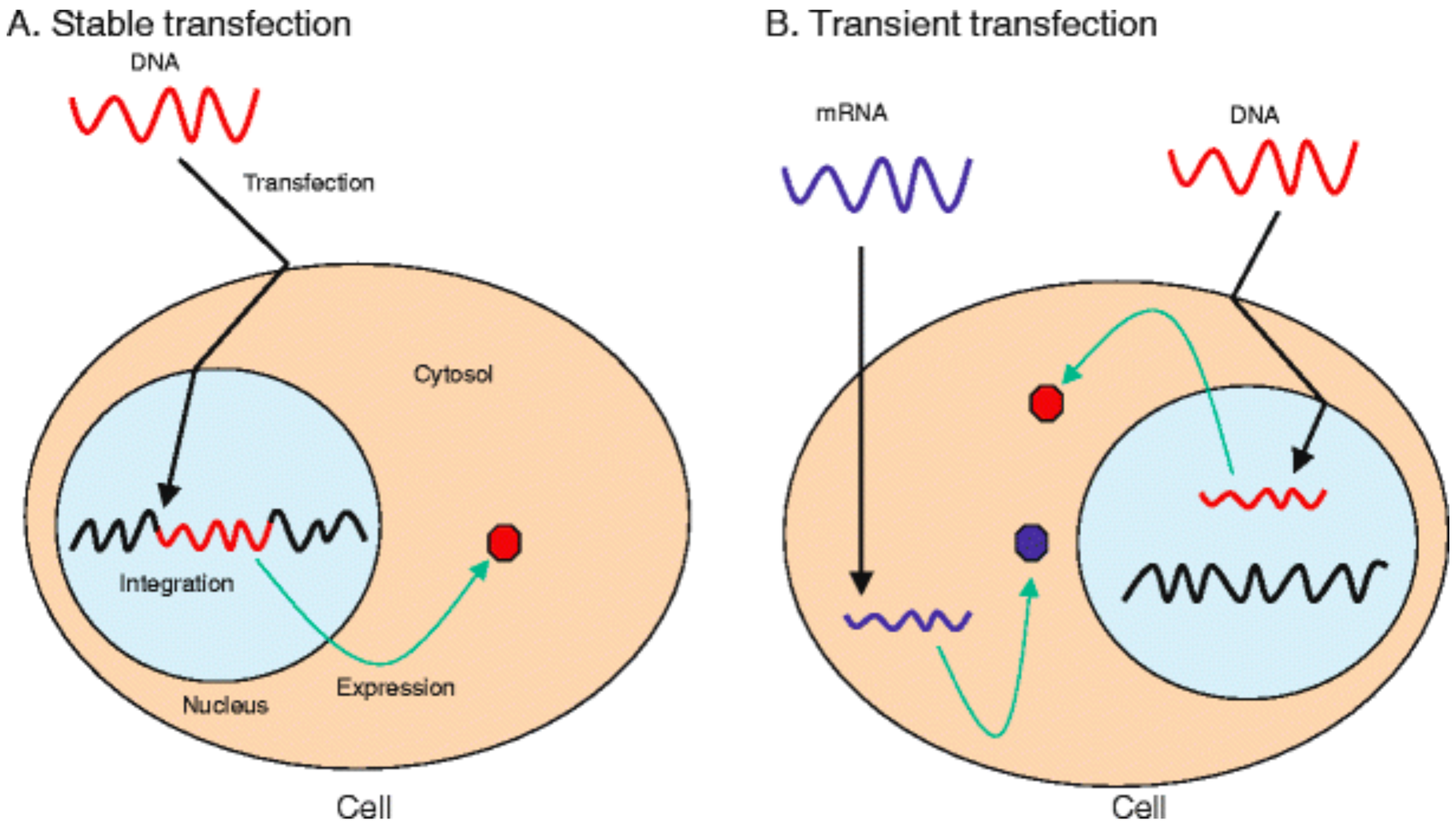
Sample	Acceptance Criteria	Results				
Drug Substance	≤ 330 ng DNA / mg RNA	17	29	10	23	211

Abbreviations: DNA = deoxyribonucleic acid; RNA = ribonucleic acid

So what?

An unknown portion of these dsDNA contaminants are replication competent plasmids that can transform *E.coli* with a simple 20 second 42C heat shock treatment. These plasmids provide antibiotic resistance on LB-Kan plates and can be isolated from *E.coli* cultures. It is unlikely these plasmids will express spike protein in non-laboratory modified *E.coli* as the ribosomal signals in the vaccine mRNA are designed for mammalian translation. The T7 promoter is known to leak in mammalian cell lines and some laboratory *E.coli* genotypes but is not expected to leak in wild type *E.coli*. This may enable mRNA to be expressed from these plasmids in mammalian cells but unless the plasmids are integrated into the human genome, they are unlikely to be replicated to high copy number.

While bacteria are unlikely to express this spike protein, bacteria can replicate this plasmid and serve as a bactofection source for introduction of these mammalian expression plasmids to human cells.


A. Stable transfection
DNA
Transfection
Cytosol
Integration
Expression
Nucleus
Cell
B. Transient transfection
mRNA
DNA
Cell

- The critiques of Alden et al focused primarily on the fact that LINE-1 is predominantly expressed in cancer cells lines and that the LINE-1 observation shouldn't be extrapolated to patients.
- The vaccines are providing trillions of dsDNAs containing a potentially leaky T7 promoter
- With these levels of contan nome integration.
- These data cannot inform wed deep sequencing work is required … in patients.
- Regardless … dsDNA cont by several orders of magnitude
- and further scrutiny should se vaccines.

Comment published on 11 April 2022, see *Curr. Issues Mol. Biol.* **2022**, *44*(4), 1661-1663.

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Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line

by Markus Aldén [1], Francisko Olofsson Falla [1], Daowei Yang [1], Mohammad Barghouth [1], Cheng Luan [1], Magnus Rasmussen [2] and Yang De Marinis [1,*]

[1] Department of Clinical Sciences, Lund University, 20502 Malmö, Sweden
[2] Infection Medicine, Department of Clinical Sciences, Lund University, 22362 Lund, Sweden
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Curr. Issues Mol. Biol. **2022**, *44*(3), 1115-1126; https://doi.org/10.3390/cimb44030073

Received: 18 January 2022 / Revised: 19 February 2022 / Accepted: 23 February 2022 / Published: 25 February 2022

Article | Full-text available

Is There a Link between the 2021 COVID-19 Vaccination Uptake in Europe and 2022 Excess All-Cause Mortality?

January 2023

DOI: 10.21276/apjhs.2023.10.1.6

Jarle Aarstad · Olav Andreas Kvitastein

https://www.researchgate.net/publication/369204376_Is_There_a_Link_between_the_2021_COVID-19_Vaccination_Uptake_in_Europe_and_2022_Excess_All-Cause_Mortality

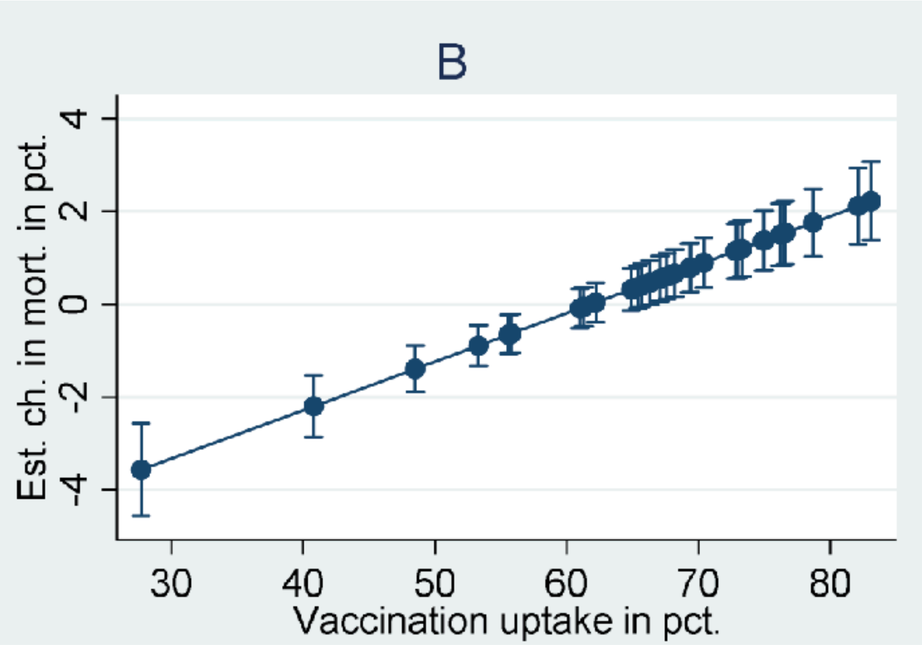
Table 3. Multi-level mixed-effects random intercept linear regressions with robust standard errors and 2022 monthly all-cause mortality compared to 2016-19 monthly averages as the dependent variable. Weighted by countries' population size.

	Model 1	Model 2	Model 3	Model 4	Model 5ª
FIXED EFFECTS					
Intercept	9.98***	9.98***	9.98***	9.98***	9.21***
	(0.439)	(0.481)	(0.423)	(0.463)	(0.470)
Vaccination uptake by the end of 2021 (V)	**0.016**	**0.050**	**0.130†**	**-0.066**	**0.004**
	(0.105)	(0.048)	(0.067)	(0.077)	(0.105)
	[5.17]	[1.00]	[2.23]	[2.76]	[5.18]
Month number in 2022 (M)	**0.716****	**0.716****	**0.716****	**0.716****	**0.484†**
	(0.262)	(0.262)	(0.249)	(0.258)	(0.280)
	[1.00]	[1.00]	[1.00]	[1.00]	[1.00]
Avg. 2020-2021 mort. rel. to 2016-2019 (A)	0.190		0.198†		0.105
	(0.137)		(0.117)		(0.146)
	[2.23]		[2.23]		[2.23]
Life expectancy 2019 (L)	0.538†			0.565	0.210
	(0.318)			(0.346)	(0.333)
	[3.77]			[3.76]	[3.77]
V*M	**0.105*****	**0.105*****	**0.090*****	**0.111****	**0.091*****
	(0.015)	(0.015)	(0.022)	(0.032)	(0.015)
	[1.00]	[1.00]	[2.23]	[3.76]	[1.01]
A*M			-0.037		
			(0.048)		
			[2.23]		
L*M				-0.031	
				(0.151)	
				[3.76]	
RANDOM EFFECTS					
Residual	38.3	38.3	38.1	38.3	28.5
	(7.88)	(7.88)	(8.08)	(7.96)	(5.62)
Country effect	1.52	2.53	2.00	2.02	2.39
	(2.43)	(2.65)	(2.59)	(2.64)	(2.31)
Wald χ^2	128.2***	134.0***	191.1***	179.5***	109.0***
Log pseudo-likelihood	-1.35e10	-1.36e10	-1.36e10	-1.36e10	-1.16e10

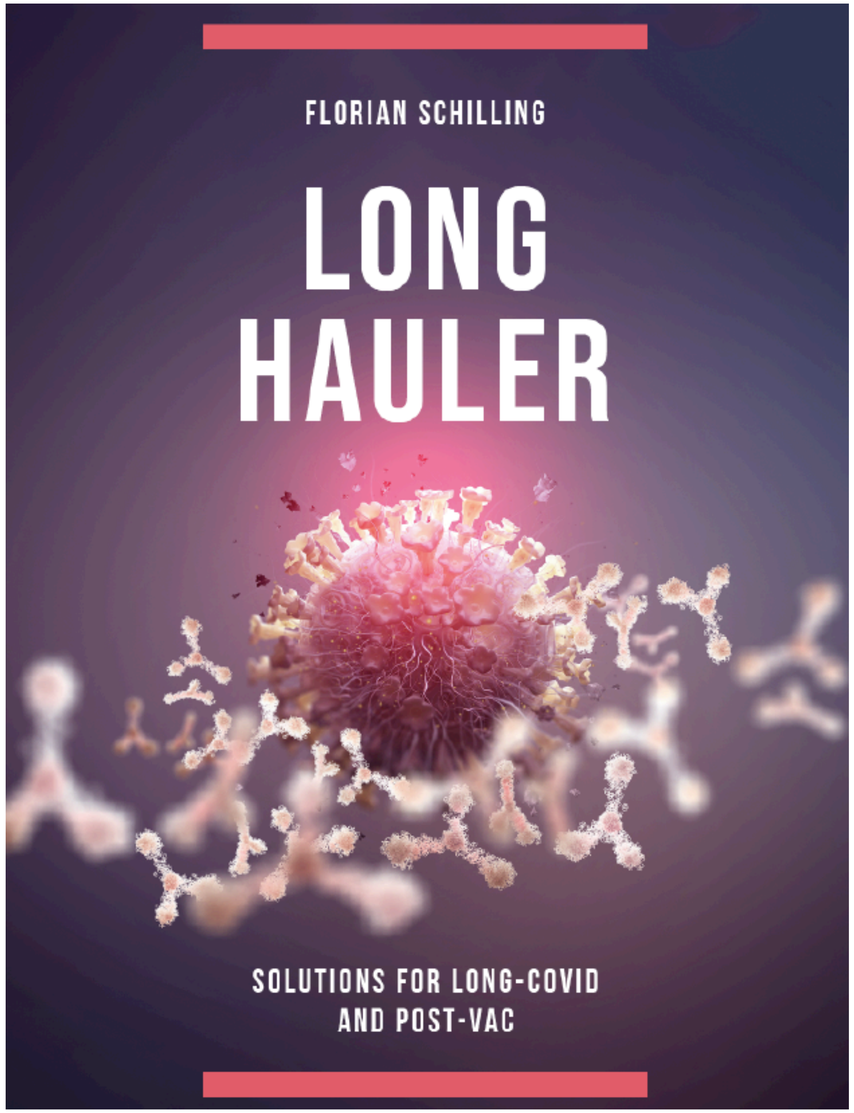
Estimates are weighted by country size in population size by January 1, 2020, and we report robust standard errors in parentheses. We report variance inflation factors (VIFs) in brackets. For fixed effects, we report conservative two-tailed tests of significance. † $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Models 1-4 have 279 monthly observations (nine monthly observations per each of the 31 countries). ªModel 5 excludes July 2022 and has 248 monthly observations (eight observations per each of the 31 countries).

- Direkte Korrelation
- Plus 1% Impfquote > Plus 0.1% Mortalität

Übersterblichkeit



- Bedeutung 0.1% Mortalität für Deutschland:
- Entspricht ca. 1000 Todesfällen / Jahr



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